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Neurosine, Its Identification with N-acetyl-L-histidine and Distribution in Aquatic Vertebrates[1]

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Introduction

Neurosine, a ninhydrin negative imidazole fraction isolated from the brain of several bony fish, amphibians and reptiles (Baslow, 1963; 1964) has been found to be similar to an imidazole fraction (IM_1) isolated by Correale (1958) from the brain of several cold-blooded vertebrates. Recent studies show that the major imidazole compound in the IM_1 fraction of the frog *Rana esculenta* is N-acetyl-L-histidine (Anastasi *et al.*, 1964). The identification of neurosine, obtained from fish brain, with N-acetyl-L-histidine and the phylogenetic and tissuc distribution of this compound in primitive and modern fishes and other aquatic vertebrates is reported.

Materials and Methods

Tissues were homogenized in a solution of 95% ethanol and 0.1 N HCl (1:1) and extracted at 6°C for two hours as described previously (Baslow, 1964). After centrifugation the clear supernatant was used for analysis.

Paper chromatograms were run with n-butanol: acetic acid: water (4 : 1 : 5); n-butanol: 1.5 M-ammonia (75 : 25), and n-butanol : acetone : water : ammonia (10 : 10 : 5 : 2) mixtures on Whatman #1 paper. The spots of the neurosine imidazole fraction and authentic N-acetyl-L-histidine (Calbiochem) were located on paper chromatograms by the Pauly reaction.

Electrophoresis experiments were run at 400-500 VDC (Beckman Duostat, cell model R, series D) in 1% acetic acid; 1% NH_4OH, and $\frac{M}{50}$ sodium borate 10 H_2O.

Hydrolysis of authentic N-acetyl-L-histidine was carried out in 6N HCl at 100°C for one hour, and enzymatically with fish brain homogenates at 24°C for 1.5 hours.

The content of N-acetyl-L-histidine in tissue samples was determined by comparison of the weight of the excised spot, after chromatography, of a known amount of authentic substance with that from a known amount of tissue extract (Lederer & Lederer, 1957). Assay values were found to be reproducible with a variation of $\pm$ 15%.

Observations and Results

A. Identification of neurosine with N-acetyl-L-histidine. Previously, the ninhydrin negative characteristic of the major imidazole present in the neurosine fraction was ascertained, in addition to its ease of conversion into a ninhydrin positive substance by hydrolysis in 6N HCl at 100°C for less than one hour or by incubation with fish brain homogenates (Baslow, 1964). In this investigation, synthetic N-acetylhistidine was found to have similar acid and tissue hydrolysis characteristics with histidine recovered as a product.

On descending paper chromatograms, both the Pauly positive spot of neurosine and N-acetylhistidine and similar R_f values of 0.31; 0.07 and 0.41 respectively in n-butanol : acetic acid : water; n-butanol : ammonia and n-butanol : acetone : water : ammonia and appear as a single spot in analysis of mixtures.

On electrophoresis, neurosine and N-acetylhistidine behaved identically. At 500 VDC for two hours in 1% acetic acid (pH 2.8), both migrate 7.9 cm. toward the cathode; at 400 VDC for two hours in $\frac{M}{50}$ sodium borate (pH 8.8), 5.2 cm. toward the anode; and 500 VDC for one hour in 1% NH_4OH (pH 10.5), 0.9 cm. toward the anode.

B. *N-acetylhistidine in brain and other tissues of primitive and modern fishes.* In previous analysis of fish brains for the presence of neurosine (N-acetyl-L-histidine), this substance could not be found in the brain of the sea lamprey, *Petro-*

[1]Supported by a grant from the John A. Hartford Foundation Inc.

myzon marinus, and the spiny dogfish, *Squalus acanthias*, although it was present in all bony fish examined (Baslow, 1964). A survey of the brains of various fishes for the presence of this compound (Table I) and its distribution within the nervous system and other tissues of the killifish, *Fundulus heteroclitus* (Table II) are reported.

C. *N-acetylhistidine content in the brain of other poikilothermic vertebrates and fish-eating mammals.* Analysis of the brain of various cold-blooded vertebrates and the rat, mouse and chick have shown that the neurosine or IM_1 fraction was present in amphibians and reptiles in addition to fish, but absent from the brain of homeothermic animals (Correale, 1958, 1964; Baslow, 1964). In Table III the results of analysis of brains and other tissues of several poikilotherms and some fish-eating mammals are presented.

TABLE I. N-ACETYLHISTIDINE IN THE BRAIN OF MODERN AND PRIMITIVE FISH SPECIES

Class	Species	Thermal Range[1]	Salinity[2]	N-acetylhistidine (μg/gram Fresh Tissue)
OSTEICHTHYES				
	Opsanus tau (toadfish)	T	M	820
	Spheroides maculatus (puffer)	T	M	975
	Opisthognathus aurifrons (jawfish)	W	M	1,920
	Pomatomus saltatrix (bluefish)	T	M	1,350
	Fundulus heteroclitus (killifish)	T	M	1,080
	Hippocampus hudsonius (seahorse)	W	M	1,870
	Brevoortia brevicaudata (menhaden)	T	M	1,970
	Chaetodon ocellatus (butterfly)	W	M	1,830
	Lobotes surinamenisis (tripletail)	W	M	1,760
	Prionotus evolans (sea robin)	T	M	1,470
	Osteoglossum bicirrhosum (arowana)	W	FW	610
	Electrophorus electricus (electric eel)	W	FW	1,000
	Malapterurus electricus (electric catfish)	W	FW	1,520
	Gymnarchus niloticus (knife-fish)	W	FW	380
	Amia calva (bowfin)	T	FW	200
	Polypterus ornatipinnis (bichir)	W	FW	430
	Calamoichthys calabaricus (reedfish)	W	FW	500
CHONDRICHTHYES				
	Mustelus canis (smooth dogfish)	T	M	
	Sphyrna tiburo (bonnetnose shark)	W	M	
	Carcharias limbatus (blacktip shark)	W	M	
	Negaprion brevirostris (lemon shark)	W	M	
	Dasyatis americana (southern stingray)	W	M	«20
	Urolophus jamaicensis (yellow stingray)	W	M	
	Hydrolagus colleii (ratfish)	Ar	M	
AGNATHA				
	Myxine glutinosa (hagfish)	Ar	M	
Lower limit of the method				20 μg/gram of tissue

[1]Temperate (T), Warm (W), Arctic (Ar).

[2]Marine (M), Fresh Water (FW).

TABLE II. N-ACETYLHISTIDINE IN THE NERVOUS SYSTEM AND OTHER TISSUES OF THE KILLIFISH *Fundulus heteroclitus*

Tissue	N-acetylhistidine (μg/gram Fresh Tissue)
Brain	
Telencephalon	950
Diencephalon	760
Mesencephalon	1,000
Metencephalon	1,000
Myelencephalon	500
Spinal cord	240
Eye	
Lens	765
Retina (pigment, chorioid and all cell layers)	165
Ocular fluid (vitreous and aqueous humors)	«10/ml
Optic nerve	90
Heart	«20
Liver	
Muscle	
Lower limit of the method	20 μg/gram of tissue

TABLE III. N-ACETYLHISTIDINE CONTENT OF THE BRAIN OF SEVERAL POIKILOTHERMS AND FISH-EATING MAMMALS.

Species	N-acetylhistidine (μg/gram Fresh Tissue)
Amphibians	
Triturus viridescens (salamander)	715
Rana pipiens (grass frog)	580
Reptiles	
Pseudemys floridana (turtle)	410
Mammals[1]	
Phoca groenlandicus (harp seal)	«20
Phoca hispida (ringed seal)	
Phoca vitulina (harbor seal)	
Delphinapterus leucas (white whale)	
Eye	
Lens	
Retina (pigment, chorioid and all cell layers)	
Ocular Fluid (vitreous humor)	
Lower limit of the method	20 μg/gram of tissue

[1]Tissues obtained 1-4 hours post-mortem.

Discussion

The distribution of N-acetyl-L- histidine in the brain of fish seems to be based upon phylogenetic relationships rather than on the basis of environmental factors such as salinity and temperature. The substance could not be found in the brain or other tissues of several fish-eating mammals even though their daily intake may reach 200 milligrams. Quantitatively, there appear to be lower concentrations of this compound in the brain of fresh water bony fish and amphibians than in marine forms. N-acetylhistidine could not be found in the brain of members of the primitive fish classes, the Agnatha and Chondrichthyes, although Correale (1958) has reported relatively large amounts of the IM_1 fraction in the shark, *Mustelus mustelus*.

Four imidazole fractions have been isolated from the brains of elasmobranchs, one of which (C_4) runs faster (R_f0.40) than N-acetylhistidine and another (C_3) which runs behind (R_f0.26) this compound in an n-butanol: acetic acid: water system (Baslow, unpublished observation). The individuality of the C_4 and C_3 components has been established by the addition of authentic N-acetylhistidine to elasmobranch brain extracts prior to chromatography. The C_2 fraction (R_f0.22), which is present in fairly high concentration, has only been isolated from members of the Chondrichthyes and may represent the major imidazole in the IM_1 fraction isolated from this group.

The presence of N-acetylhistidine in the optic nerve, retina and lens of fish may indicate an important role in visual processes. The meaning of these findings and those of Correale (1958), who found appreciable concentrations of N-acetylhistidine in frog retina, and of Anastasi *et al.* (1964) who find large quantities of this substance in amphibian lens is, however, obscure. Ninhydrin-negative or faintly ninhydrin-positive substances also reported in the N-acetylhistidine fraction are probably the source of amino acid residues isolated upon acid hydrolysis of the neurosine fraction (Baslow, 1964). It is suggested that the presence of this substance is associated with development of higher order central nervous control and integration of senses typical of bony fish and their descendants, the tetrapoda.

Summary

Neurosine, a ninhydrin-negative imidazole fraction, isolated from the brain of cold-blooded vertebrates, has been identified with N-acetyl-L-histidine. This compound has been found in the brain of bony fish (Osteichthyes) but could not be identified in the brain of more primitive fish, the cyclostomes (Agnatha) and sharks, rays and chimeras (Chondrichthyes).

N-acetylhistidine has been found in high concentration in the lens, optic lobes (mesencephalon) and cerebellum (metencephalon) and in lower concentration in the retina and other portions of the brain and spinal cord, but not in other bony fish tissues. It has been found in the brain of bony fish living under all conditions of physical activity, inhabiting environments including marine and fresh waters and living in thermal environments including tropical and temperate waters.

***Since this article went to Press, Erspamer *et al.* (*Journ. Neurochem.*, Vol. 12, Pt. 2, pp. 123-130, 1965) have suggested that neurosine and N-acetyl-L-histidine are identical, and have confirmed the absence of this compound in elasmobranchs.

Acknowledgments

I am indebted to M. F. Stempien of the Department of Marine Biochemistry and Ecology, New York Zoological Society, and H. W. Graham of the Bureau of Commercial Fisheries, Woods Hole, for the procurement of brains of sharks and rays; to L. Margolis and J. A. Thomson of the Fisheries Research Board of Canada, Nanaimo, for chimera brains; and to P. Montreuil and C. Ray of the New York Aquarium for hagfish, seal and whale brains.

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